

Table S1: Biochemical properties of *in vitro* Dps binding to linear DNA sequences containing the promotor sequences *recA* (252 bp), *fluP* (401 bp), λ P_R (483 bp), and *rrnB* P1 (120 bp). Mean apparent K_D and mean Hill coefficients were calculated based on the analysis of gel-shift assays performed for each DNA template. Related to **Figure 3**.

Template	Mean apparent $K_D \pm \text{SEM}$ [μM]	Mean Hill Coef. $\pm \text{SEM}$
<i>recA</i>	0.54 ± 0.06	3.33 ± 0.31
<i>rrnB</i> P1	0.57 ± 0.04	2.97 ± 0.17
<i>flu</i>	0.64 ± 0.03	3.10 ± 0.11
λ P _R	0.77 ± 0.04	3.29 ± 0.18

Table S2: Statistics of single-molecule transcription experiments. Denoted are the number of traces, dwell times measured, average elongation rates, and pause probabilities (SP = short pause lengths between 1 - 5 s; LP = long pause lengths between 5 – 100 s), respectively, for each type of single-molecule transcription experiment. Related to **Figure 5**.

	AF	AF + Dps	OF	OF + Dps
<i>N</i>	76	47	49	38
Dwell times	12,839	7,341	5,687	4,339
Elongation rate [nt/s]	26.6 ± 0.3	26.3 ± 0.4	23.5 ± 0.3	22.7 ± 0.5
SP probability	0.36 ± 0.006	0.37 ± 0.008	0.44 ± 0.01	0.43 ± 0.01
LP probability	0.024 ± 0.003	0.025 ± 0.003	0.038 ± 0.005	0.029 ± 0.005

Table S3: Primer sequences used for the production of DNA tether constructs. Related to STAR Methods section.

Oligonucleotide	Sequence
1	5'- GACCGAGATAGGGTTGAGTG
2	5'- CCATCTTGGTCTCCCTACGCTCTAGAACTAGTGGATCCCC
3	5'- CCATCTTGGTCTCCTAGGCGTCAGCCTGCGAAGCAGTGGC
4	5'- CCATCTTGGTCTCCTGTCAACACCACCTTGCTCCGAGGTT
5	5'- CCATCTTGGTCTCCGACAGCGCCATTGCCATTAGGCTG
6	5'- CTTCTGCTTCCTGATGCAAAAAC
7	5'- CTGCGGTCTCGCCCACCGGCTCCAGATTATCAGC
8	5'- CTGCGGTCTCGTCAAAACTGGAACAACACTCAACCC
9	5'- CTGCGGTCTCGTAGGAGGCGCCATTGCCATTAGG
10	5'- CTGCGGTCTCGCCGGGTTGCAGCACTGGGCCAGATG
11	5'- GACCGAGATAGGGTTGAGTG
12	5'- CAGGGTCGGAACAGGGAGAGC
13	5'- CTGCGGTCTCGCCCACCGGCTCCAGATTATCAGC
14	5'- CTGCGGTCTCGTCAATTACGCGCAGCGTACCGCTAC
15	5'- CTGCGGTCTCGTAGGCCATTAGAGCTTGACGGGG
16	5'- CTGCGGTCTCGCCGGGTTGCAGCACTGGGCCAGATG

Table S4: Primer sequences used for the production of DNA promoter templates. Related to STAR Methods section.

Oligonucleotide	Sequence
<i>rrnB</i> P1 pIA536	5'- TCAGGAATTCTAACCGCGGTCAAGAAAATTATTTT
<i>rrnB</i> P1 pIA536	5'- GTTCGGCATGGGTCAGGTG
λ Pr pIA1240	5'- CGTTAAATCTATCACCGCAAGG
λ Pr pIA1240	5'- CAGTTCCCTACTCTCGCATG
<i>recA</i> pIA1222	5'- GTAAAACGACGGCCAGT
<i>recA</i> pIA1222	5'- ATTACGAATTGCGAGAGACATCG
<i>Pflu</i> <i>E. coli</i> genomic DNA	5'- ATTTCTTCTGAACCTGTCGTG
<i>Pflu</i> <i>E. coli</i> genomic DNA	5'- ACGAAAGCGCCCGTCATGTG